

## An evaluation of EDTA compounds for iron fortification of cereal-based foods

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Fe absorption was measured in adult human subjects consuming different cereal foods fortified with radiolabelled FeSO<sub>4</sub>, ferrous fumarate or NaFeEDTA, or with radiolabelled FeSO<sub>4</sub> or ferric pyrophosphate in combination with different concentrations of Na<sub>2</sub>EDTA. Mean Fe absorption from wheat, wheat–soyabean and quinoa (*Chenopodium quinoa*) infant cereals fortified with FeSO<sub>4</sub> or ferrous fumarate ranged from 0.6 to 2.2 %. For each infant cereal, mean Fe absorption from ferrous fumarate was similar to that from FeSO<sub>4</sub> (absorption ratio 0.91–1.28). Mean Fe absorption from FeSO<sub>4</sub>-fortified bread rolls was 1.0 % when made from high-extraction wheat flour and 5.7 % when made from low-extraction wheat flour. Fe absorption from infant cereals and bread rolls fortified with NaFeEDTA was 1.9–3.9 times greater than when the same product was fortified with FeSO<sub>4</sub>. Both high phytate content and consumption of tea decreased Fe absorption from the NaFeEDTA-fortified rolls. When Na<sub>2</sub>EDTA up to a 1:1 molar ratio (EDTA:Fe) was added to FeSO<sub>4</sub>-fortified wheat cereal and wheat–soyabean cereal mean Fe absorption from the wheat cereal increased from 1.0 % to a maximum of 5.7 % at a molar ratio of 0.67:1, and from the wheat–soyabean cereal from 0.7 % to a maximum of 2.9 % at a molar ratio of 1:1. Adding Na<sub>2</sub>EDTA to ferric pyrophosphate-fortified wheat cereal did not significantly increase absorption ( $P > 0.05$ ). We conclude that Fe absorption is higher from cereal foods fortified with NaFeEDTA than when fortified with FeSO<sub>4</sub> or ferrous fumarate, and that Na<sub>2</sub>EDTA can be added to cereal foods to enhance absorption of soluble Fe-fortification compounds such as FeSO<sub>4</sub>.

### Iron fortification: Iron absorption: EDTA: Cereal-based foods

Fe deficiency is a major cause of anaemia in infants, children, and women of reproductive age, especially in the poorer countries of the developing world, and to a lesser extent in the more industrialised nations, (DeMaeyer & Adiels-Tegman, 1985). Fe-deficiency anaemia can decrease mental and psychomotor development in children (Lozoff *et al.* 1991), increase both morbidity and mortality of mother and child at childbirth, decrease work performance, and decrease resistance to infection (Scrimshaw, 1984; Hercberg *et al.* 1987). One strategy to prevent Fe-deficiency anaemia is to fortify food products with Fe; cereal-based foods are a popular choice as vehicles for Fe fortification. Cereal flours, such as wheat and maize, are fortified with Fe to supplement the general population,

whereas Fe-fortified infant cereals and breakfast cereals are targeted more specifically at infants, children and adolescents (Hurrell, 1997).

Cereal-based foods, however, are particularly difficult to fortify with Fe, since they contain significant quantities of phytic acid, a potent inhibitor of Fe absorption (Hallberg *et al.* 1987; Hurrell *et al.* 1992). In addition, when soluble Fe compounds of high relative bioavailability, such as FeSO<sub>4</sub> are added, cereal foods readily become rancid during storage due to Fe-catalysed fat oxidation reactions (Hurrell *et al.* 1989). As a result of these potential organoleptic problems, many cereal-based foods are fortified with elemental Fe powders which, at best, are only about half as well absorbed as FeSO<sub>4</sub> (Hurrell, 1997).

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Some infant cereals are now fortified with ferrous fumarate, which is reported to have equivalent absorption to  $\text{FeSO}_4$  with less sensory problems (Hurrell *et al.* 1989).

The most effective way to overcome the inhibitory effect of phytic acid and to promote absorption of fortification Fe is to add ascorbic acid (Forbes *et al.* 1989; Davidsson *et al.* 1994a). Without the addition of ascorbic acid to Fe-fortified cereals, Fe absorption may be as low as 1 %, even when  $\text{FeSO}_4$  is added (Derman *et al.* 1980; Cook *et al.* 1997). The problem with ascorbic acid is that it is readily degraded during food processing (Hallberg *et al.* 1989) and during storage if the food is not in a special package designed to keep out  $\text{O}_2$  and humidity. Although ascorbic acid is often added to Fe-fortified cereals as an absorption enhancer, it has not been added to cereal flours.

An alternative Fe compound that appears highly suitable for the fortification of cereal-based foods is  $\text{NaFeEDTA}$ . This compound has been reported to be about two to four times better absorbed than  $\text{FeSO}_4$  from a variety of meals containing cereals and legumes (Layrisse *et al.* 1977; Martinez-Torres *et al.* 1979; MacPhail *et al.* 1981). Furthermore, it does not promote fat oxidation in stored wheat flour (Hurrell, 1997), and it is stable during processing and storage. It would also seem possible to add the EDTA moiety alone as an absorption enhancer in combination with other Fe compounds. When El-Guindi *et al.* (1988) added equimolar quantities of  $\text{FeSO}_4$  and  $\text{Na}_2\text{EDTA}$  to Egyptian bread, Fe absorption increased from 2.1 % to 5.3 %. More recently, MacPhail *et al.* (1994) reported a 3-fold increase in Fe absorption from a  $\text{FeSO}_4$ -fortified rice meal when  $\text{Na}_2\text{EDTA}$  was added at a EDTA:Fe molar ratio of 0.5:1, compared with a 2-fold increase at a molar ratio of 1:1.

In the present study, we have used a radio-Fe technique to measure Fe absorption by adult human subjects from a selection of infant cereals and wheat bread rolls fortified with  $^{59}\text{FeSO}_4$ , [ $^{55}\text{Fe}$ ]ferrous fumarate or  $\text{Na}^{55}\text{FeEDTA}$ . In addition, we have investigated the absorption-enhancing effect of different concentrations of  $\text{Na}_2\text{EDTA}$  added to  $^{59}\text{FeSO}_4$ - and [ $^{55}\text{Fe}$ ]ferric pyrophosphate-fortified wheat infant cereal and to  $^{59}\text{FeSO}_4$ -fortified wheat-soyabean infant cereal.

## Subjects, methods and materials

### Subjects

Fe absorption was measured in eighty-four volunteer subjects aged 18–40 years. The total group included thirty-six males and forty-eight females. All subjects were in good health and denied a history of disorders known to influence the gastrointestinal absorption of Fe. Serum ferritin concentrations ranged from 6 to 668  $\mu\text{g/l}$ , indicating a wide variation in Fe status. Fifteen of the subjects, one male and fourteen females, were Fe deficient as defined by a serum ferritin concentration  $<12 \mu\text{g/l}$ . Written informed consent was obtained from each volunteer before the investigation, and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center. Subjects were

allocated to the studies in the order which they volunteered. There was no randomisation by gender or Fe status.

### Iron absorption measurements

Nine Fe absorption studies were carried out, during which three to four separate Fe absorption measurements were performed in each of seven to ten subjects by using radio-Fe tracers administered sequentially. All meals were administered between 07.00 and 09.00 hours after an overnight fast and water only was allowed for 3 h. The test meals were fed with labelled Fe compounds providing either 37 kBq  $^{59}\text{Fe}$  or 74 kBq  $^{55}\text{Fe}$ , and Fe absorption was measured based on erythrocyte enrichment as previously described (Cook *et al.* 1972).

On the day preceding administration of the first test meal, 30 ml blood was collected from each subject in an EDTA-treated tube for measurement of packed cell volume, serum ferritin (Flowers *et al.* 1986) and background radioactivity. Meals A (labelled with  $^{55}\text{Fe}$ ) and B (labelled with  $^{59}\text{Fe}$ ) were fed on days 2 and 3 of the study respectively. At 14 d after administration of meal B (day 17), 30 ml blood was drawn for the measurement of incorporated erythrocyte radioactivity. In studies nos. 1–5, 7 and 8, test meals C and D tagged with separate radio-Fe labels were fed on days 17 and 18 respectively, and a final blood sample was obtained on day 32 to determine the increase in erythrocyte radioactivity. In study no. 6, only meal C was fed; a final blood sample was collected on day 31. Measurements of blood radioactivity were performed on duplicate 10 ml samples of whole untreated blood by a modification of the method of Eakins & Brown (1966). Briefly, after digesting whole blood in  $\text{HNO}_3$ , Fe is precipitated twice with  $\text{NH}_4\text{OH}$  and redissolved in  $\text{H}_3\text{PO}_4$  before finally precipitating with  $\text{NH}_4\text{Cl}$  and ethanol and suspending the precipitate in a gel with a scintillation fluor for counting (Bothwell *et al.* 1979). Percentage absorption was calculated on the basis of blood volume estimated from height and weight (Wennesland *et al.* 1959; Brown *et al.* 1962) and an assumed erythrocyte incorporation of 80 % (Hosein *et al.* 1967).

### Radioactive iron compounds

The Fe fortification compounds  $\text{NaFeEDTA}$ , ferrous fumarate and ferric pyrophosphate were labelled with  $^{55}\text{Fe}$ . They were synthesised in 10 g batches using a scaled-down version of the normal manufacturing procedures (Dr Paul Lohman Co., Emmerthal, Germany). The radioactive Fe compounds were in crystalline form and had similar appearance, particle size and solubility in dilute HCl to their commercial counterparts. The measured Fe content of  $\text{Na}^{55}\text{FeEDTA}$  was 16.2 % and it contained 15.9 kBq/mg Fe. The measured Fe content of [ $^{55}\text{Fe}$ ]ferrous fumarate was 32.2 % and it contained 20.4 kBq/mg Fe. The measured Fe content of [ $^{55}\text{Fe}$ ]ferric pyrophosphate was 28 % and it contained 23.8 kBq/mg Fe. Non-radioactive Fe compounds were provided by the same manufacturer.

**Table 1.** Iron absorption from cereal-based foods fortified with NaFeEDTA, ferrous sulfate and ferrous fumarate in adult human subjects†

Study no.	Study design	Serum ferritin (µg/l)		Test meals	Fe absorption (% dose)			Absorption ratio§		
		Mean‡	Range		Mean‡	–1 SE	+1 SE	Mean‡	–1 SE	+1 SE
1	Wheat infant cereal (3 M, 5 F, 22 years)	39	10–178	A Ferrous fumarate	2.06	1.48	2.88	0.94	0.78	1.13
				B Unfortified	3.90	2.60	5.87	1.78*	1.41	2.23
				C NaFeEDTA	5.23	4.02	6.79	2.37***	2.19	2.58
				D FeSO <sub>4</sub>	2.20	1.66	2.91	–	–	–
2	Wheat–soy infant cereal (4 M, 5 F, 27 years)	42	13–104	A Ferrous fumarate	0.93	0.62	1.40	1.28	0.99	1.66
				B Unfortified	1.13	0.76	1.69	1.56*	1.40	1.74
				C NaFeEDTA	2.81	1.91	4.13	3.86***	3.25	4.59
				D FeSO <sub>4</sub>	0.73	0.49	1.07	–	–	–
3	Quinoa ( <i>chenopodium quinoa</i> ) infant cereal (3 M, 4 F, 22 years)	39	20–118	A Ferrous fumarate	0.57	0.47	0.70	0.91	0.67	1.25
				B Unfortified	0.67	0.47	0.96	1.07	0.86	1.35
				C NaFeEDTA	1.68	1.25	2.25	2.60**	2.28	3.14
				D FeSO <sub>4</sub>	0.63	0.42	0.94	–	–	–
4	Wheat–soyabean infant cereal (4 M, 5 F, 23 years)	41	11–114	A NaFeEDTA	3.32	2.40	4.60	3.53**	3.00	4.01
				B FeSO <sub>4</sub>	0.94	0.70	1.26	–	–	–
				C NaFeEDTA × 3	1.94	1.60	2.34	2.70**	1.01	4.25
				D FeSO <sub>4</sub> × 3	0.72	0.50	1.04	–	–	–
5	Low-extraction wheat bread roll (5 M, 5 F, 25 years)	54	13–668	A NaFeEDTA	11.50	8.62	15.4	2.02**	1.72	2.38
				B FeSO <sub>4</sub>	5.70	4.30	7.55	–	–	–
				C NaFeEDTA, tea	1.86	1.30	2.65	1.81**	1.57	2.10
				D FeSO <sub>4</sub> , tea	1.03	0.69	2.65	–	–	–
6	High-extraction wheat bread roll (6 M, 4 F, 26 years)	20	3–60	A Unfortified	2.27	1.58	3.27	2.29*	1.67	3.14
				B NaFeEDTA	3.91	2.68	5.69	3.94***	3.28	4.74
				C FeSO <sub>4</sub>	0.99	0.72	1.36	–	–	–

M, male; F, female.

Mean values were significantly different from 1: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

† For details of procedures, see p. 904.

‡ Geometric mean.

§ Absorption from test meal compared with absorption from FeSO<sub>4</sub> (studies nos. 1–6), FeSO<sub>4</sub> × 3 (study no. 4) or FeSO<sub>4</sub> plus tea (study no. 5) test meals.

### Cereal-based foods

Three experimental infant cereals were prepared at the Nestlé Product Technology Centre, Orbe, Switzerland. The wheat-based infant cereal was made from 60 % extraction wheat flour and the quinoa-based infant cereal was made from ground whole sweet quinoa (*Chenopodium quinoa*) grains (Nestlé Development Centre, Quito, Ecuador). The flours were mixed with sucrose–water (1:10, w/v) to reach a slurry with about a 40 % (w/v) DM. The slurry was cooked by steam injection (about 135°C) and roller-dried. No other ingredients were added. The wheat–soyabean-based infant cereal was prepared in a similar way, but from a mixture of 60 %-extraction wheat flour and soyabean-protein isolate (approximately 6:1, w/w). The protein content (N × 6.25; %, w/w) was 12.3 for wheat cereal, 12.9 for quinoa cereal and 17.2 for wheat–soyabean cereal. Phytic acid was measured by a modification of the Makover (1970) method in which Ce replaced Fe in the precipitation step. Phytic acid (mg/100 g) was 122, 763 and 770 respectively for the wheat, quinoa and wheat–soyabean cereals.

Two different bread rolls were prepared and baked in the laboratory. A low-extraction roll was prepared from the same 60 %-extraction wheat flour as the wheat infant cereal. Salt, sugar, yeast, water and the radioactive Fe compounds were added during preparation of the dough. One series of low-extraction wheat rolls was fortified with Na<sup>55</sup>FeEDTA and the other with <sup>59</sup>FeSO<sub>4</sub>. Each roll contained 35 g wheat flour, 2.15 mg Fe and either 55.5 kBq <sup>55</sup>Fe or 18.5 kBq <sup>59</sup>Fe. A high-extraction-wheat roll was prepared in a similar way from 80 %-extraction

wheat flour (Nestlé Product Technology Centre). The rolls were either fortified with Na<sup>55</sup>FeEDTA or <sup>59</sup>FeSO<sub>4</sub> or were unfortified. Each roll contained 50 g wheat flour and the Fe-fortified rolls contained 2.5 mg Fe either as Na<sup>55</sup>FeEDTA (37 kBq) or <sup>59</sup>FeSO<sub>4</sub> (18.5 kBq). Phytic acid was degraded to zero during the preparation of the low-extraction-wheat roll, but was not analysed in the high-extraction-wheat roll.

### Test meals

In studies nos. 1–3 (Table 1) Fe absorption was compared in subjects fed the infant cereals either unfortified, or fortified with NaFeEDTA, ferrous fumarate or FeSO<sub>4</sub>. All test meals contained 50 g infant cereal, 10 g sucrose and 0.5 g salt, and were mixed into a porridge with 300 ml hot water. Non-fat milk powder (Carnation, Los Angeles, CA, USA) was added to the wheat and quinoa cereals to equilibrate the crude protein (N × 6.25) to that of the wheat–soyabean cereal. Na<sup>55</sup>Fe EDTA and [<sup>55</sup>Fe]ferrous fumarate were accurately weighed to provide 74 kBq to each subject. The Fe compounds were carefully mixed into the cereal porridge together with the necessary amount of the respective non-radioactive Fe compound to provide a total of 5 mg fortification Fe to each subject. FeSO<sub>4</sub> (5 mg) was added in a slightly different way. A non-radioactive portion providing 2.5 mg Fe was carefully mixed into the cereal porridge together with a 1 ml solution containing 2.5 mg FeSO<sub>4</sub> providing 37 kBq <sup>59</sup>Fe in 0.01 mol HCl/l. The radioactive tag was added to the unfortified cereals as a 1 ml solution containing 0.1 mg Fe as FeCl<sub>3</sub> with 37 kBq

**Table 2.** Iron absorption from infant cereals fortified with ferrous sulfate and different levels of Na<sub>2</sub>EDTA in adult human subjects†

Study no.	Study design	Serum ferritin (μg/l)		Test meals (EDTA:Fe molar ratios)	Fe absorption (% dose)			Absorption ratios compared with								
								Meal A			Meal B			Meal C		
		Mean‡	SE		SE	Mean‡	SE	SE	Mean‡	SE	SE	Mean‡	SE	SE		
7	Wheat infant cereal (5 M, 5 F, 25 years)	21	6–77	A 0:1:0	1.02	0.76	1.36	—	—	—	0.40*	0.33	0.49	0.18**	0.16	0.20
				B 0.33:1:0	2.53	1.65	3.88	2.49*	2.04	3.03	—	—	—	1.44*	0.36	0.55
				C 0.67:1:0	5.71	4.22	7.73	5.62**	4.90	6.50	2.26*	1.81	2.81	—	—	—
				D 1.0:1:0	5.59	3.96	7.89	5.51**	4.58	6.63	2.21*	1.79	2.74	0.98	0.87	1.10
8	Wheat–soyabean infant cereal (3 M, 7 F, 24 years)	24	11–89	A 0:1:0	0.70	0.48	1.02	—	—	—	0.40*	0.32	0.49	0.32**	0.26	0.39
				B 0.33:1:0	1.76	1.32	2.34	2.52*	2.03	3.11	—	—	—	0.80	0.66	0.96
				C 0.67:1:0	2.22	1.64	3.00	3.17**	2.57	3.90	1.26	1.04	1.52	—	—	—
				D 1.0:1:0	2.86	2.07	3.95	4.08**	3.19	5.21	1.62*	1.33	1.97	1.29	1.01	1.65

M, male; F, female

Mean values were significantly different from one: \**P* < 0.05, \*\**P* < 0.01.

† For details of procedures, see p. 904.

‡ Geometric mean.

of <sup>59</sup>Fe in 0.01 mol HCl/l. In order to ensure complete ingestion of the radio Fe tag, after consumption of the meal the cereal bowls were carefully rinsed with water and the rinsing water consumed.

In study no. 4 (Table 1) Fe absorption was compared in subjects fed the wheat–soyabean cereal fortified with either 5 or 15 mg Fe as NaFeEDTA or FeSO<sub>4</sub>. The test meals were prepared as before. Na<sup>55</sup>FeEDTA was accurately weighed to provide 74 kBq <sup>55</sup>Fe to each subject and was carefully mixed into the cereal porridge together with sufficient non-radioactive NaFeEDTA to provide a total of 5 or 15 mg Fe per meal. FeSO<sub>4</sub> was added to the cereal as a 1 ml solution containing 2.5 mg FeSO<sub>4</sub> and 37 kBq <sup>59</sup>Fe in 0.01 mol HCl/l together with either 2.5 or 12.5 mg non-radioactive FeSO<sub>4</sub> to provide a total of 5 or 15 mg Fe per meal.

Study no. 5 (Table 1) compared Fe absorption from subjects fed two low-extraction-wheat rolls fortified with either NaFeEDTA or FeSO<sub>4</sub> and fed with or without tea. The rolls were fed with 10 g butter. For the subjects receiving tea, 3 g Assam tea purchased locally in Kansas City was infused for 10 min in 300 ml boiling water, strained and served with 10 g sugar. Subjects not consuming tea had free access to water. Study no. 6 compared Fe absorption from subjects fed two high-extraction-wheat rolls either unfortified, or fortified with NaFeEDTA or

FeSO<sub>4</sub>. The rolls were fed with 20 g butter and 200 ml water.

Studies nos 7 and 8 (Table 2) investigated the influence of different levels of Na<sub>2</sub>EDTA (Fisher Scientific, Fair Lawn, NJ, USA) in subjects fed FeSO<sub>4</sub>-fortified wheat cereal or wheat–soyabean cereal respectively. In study no. 7, each subject received 50 g wheat cereal, 10 g sucrose, 0.5 g salt and 4 mg Fe as FeSO<sub>4</sub> mixed into a porridge with 300 ml hot water. Na<sub>2</sub>EDTA and the radio-Fe tracers were mixed into the porridge just before feeding. Meals A, B, C and D contained an EDTA:Fe molar ratio of 0:1, 0.33:1, 0.67:1 and 1:1 respectively. The radio-Fe tracers were added in a 1 ml solution containing 0.1 mg Fe as FeCl<sub>3</sub> in 0.01 mol HCl/l (74 kBq <sup>55</sup>Fe to meals A and C and 37 kBq <sup>59</sup>Fe to meals B and D). Study no. 8 was identical to study no. 7 except that wheat–soyabean cereal replaced wheat cereal.

Study no. 9 (Table 3) compared Fe absorption from wheat infant cereal fortified with FeSO<sub>4</sub> or [<sup>55</sup>Fe]ferric pyrophosphate in the presence or absence of Na<sub>2</sub>EDTA. Each subject received 50 g cereal, 10 g sucrose, 0.5 g salt and 4 mg Fe, either as FeSO<sub>4</sub> or [<sup>55</sup>Fe]ferric pyrophosphate (74 kBq <sup>55</sup>Fe), mixed into a porridge with 300 ml water. FeSO<sub>4</sub> was added together with a 1 ml solution containing 0.1 mg Fe as FeCl<sub>3</sub> in 0.01 mol HCl/l providing 37 kBq <sup>59</sup>Fe. Na<sub>2</sub>EDTA was mixed into the porridge before feeding at an EDTA:Fe molar ratio of 1:1.

**Table 3.** Influence of Na<sub>2</sub>EDTA on iron absorption from ferrous sulfate- and ferric pyrophosphate-fortified wheat infant cereals in adult human subjects†

Study no.	Study design	Serum ferritin (µg/l)		Test meals	Fe absorption (% dose)			Absorption ratios with and without Na <sub>2</sub> EDTA		
		Mean‡	Range		Mean‡	–1 SE	+1 SE	Mean‡	–1 SE	+1 SE
9	Wheat infant cereal (3 M, 8 F, 24 years)	29	9–87	A Ferric pyrophosphate	0.26	0.16	0.42	—	—	—
				B FeSO <sub>4</sub>	1.76	1.29	2.39	—	—	—
				C Ferric pyrophosphate plus Na <sub>2</sub> EDTA§	0.44	0.36	0.52	1.68	1.15	2.47
				D FeSO <sub>4</sub> plus Na <sub>2</sub> EDTA§	5.93	4.66	7.54	3.37***	2.78	4.10

M, male; F, female.

Mean value was significantly different from 1: \*\*\**P* < 0.001.

† For details of procedures, see p. 904.

‡ Geometric mean.

§ Na<sub>2</sub>EDTA added at an EDTA:Fe molar ratio of 1:1.



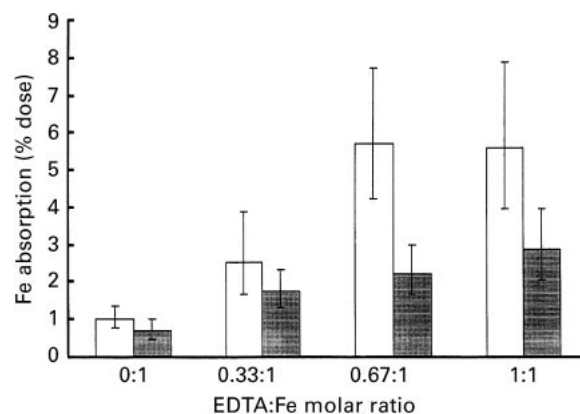
### Statistical analysis

Percentage absorption values were converted to logarithms for calculating geometric means and for statistical analysis. Original values were recovered by reconvertng the results as antilogarithms (Layrisse *et al.* 1969). Comparison of Fe absorption for any given pair of test meals within each study was made by a paired *t* test to determine whether the log absorption ratio differed from zero. The mean absorption ratios for different studies were analysed by ANOVA and significant differences between groups were determined by the Tukey's multiple comparison test (Graphpad Prism, San Diego, CA, USA). In all cases,  $P \leq 0.05$  was taken to indicate a significant difference.

### Results

Fe absorption from ferrous fumarate-, NaFeEDTA-, and FeSO<sub>4</sub>-fortified infant cereals (studies nos. 1–3) is shown in Table 1. Mean values are given for serum ferritin and percentage Fe absorption. In most cases, individual Fe absorption values were highest in those subjects with the lowest serum ferritin values. Mean Fe absorption from infant cereals containing 5 mg fortification Fe ranged from 0.57–5.23 % in the adult subjects. Absorption was higher in subjects fed the wheat cereal than in those fed the wheat–soyabean cereal, and was lowest in subjects fed the quinoa cereal. In all three cereals, ferrous fumarate was absorbed to the same extent as FeSO<sub>4</sub>. The absorption ratios, which represent the relative absorption or relative bioavailability of ferrous fumarate compared with FeSO<sub>4</sub>, were 0.91, 0.94 and 1.28 in quinoa, wheat and wheat–soyabean cereals respectively. These values were not significantly different from 1 ( $P > 0.05$ ). Fe absorption from the NaFeEDTA-fortified cereals was 2–4-fold higher than from the FeSO<sub>4</sub>-fortified cereals. Absorption ratios were 2.37, 2.60 and 3.86 respectively for the wheat, quinoa and wheat–soyabean cereals. These values were all significantly different from 1 ( $P < 0.001$ ), but not significantly different from each other ( $P > 0.05$ ; ANOVA with Tukey's multiple comparison test). Percentage Fe absorption was higher in subjects fed the unfortified cereals than in subjects fed the FeSO<sub>4</sub>-fortified wheat or wheat–soyabean cereals (absorption ratios 1.78 and 1.56 respectively;  $P < 0.05$ ) but not the quinoa cereal. Increasing the Fe content in the fortified cereals from 5 to 15 mg per meal slightly decreased Fe absorption (study no. 4, Table 1) although the absorption enhancing effect of NaFeEDTA compared with FeSO<sub>4</sub> was similar at both Fe concentrations (absorption ratios 3.53 and 2.70 respectively;  $P > 0.05$ ).

In studies nos. 5 and 6 (Table 1), Fe absorption was compared in subjects fed wheat bread rolls fortified with either NaFeEDTA or FeSO<sub>4</sub>. Mean Fe absorption ranged from 0.99 % with the FeSO<sub>4</sub>-fortified high-extraction-wheat roll to 11.5 % with the low-extraction-wheat roll fortified with NaFeEDTA. As in the previous studies, Fe absorption was two to four times higher in subjects consuming the rolls fortified with NaFeEDTA than in subjects consuming rolls fortified with FeSO<sub>4</sub>. With the low-extraction-wheat rolls (study no. 5), the absorption



**Fig. 1.** Iron absorption in adult human subjects from wheat (□; study no. 7) and wheat–soyabean (■; study no. 8) infant cereals fortified with ferrous sulfate plus different levels of Na<sub>2</sub>EDTA. Values are geometric means with 1 SE represented by vertical bars. For details of procedures, see p. 904. Adding Na<sub>2</sub>EDTA to ferrous sulfate-fortified wheat cereal up to an EDTA:Fe molar ratio of 1:1 increased absorption from 1.0 % to 5.7 %.

ratio for NaFeEDTA compared with FeSO<sub>4</sub> was 2.02 ( $P < 0.01$ ) when consumed with water and 1.81 ( $P < 0.01$ ) when consumed with tea. With the high-extraction-wheat roll, the absorption ratio was 3.94 when consumed with water ( $P < 0.001$ ). Tea consumed with the low-extraction-wheat roll significantly decreased the absorption of FeSO<sub>4</sub> ( $P < 0.001$ ) and NaFeEDTA ( $P < 0.0001$ ).

In studies nos. 7 and 8 (Table 2; Fig. 1), Fe absorption was compared in subjects fed infant cereals fortified with FeSO<sub>4</sub> with increasing amounts of Na<sub>2</sub>EDTA as an absorption enhancer. With wheat infant cereal (study no. 7), Na<sub>2</sub>EDTA increased Fe absorption more than 5-fold, from 1.02 % with FeSO<sub>4</sub> alone to a maximum of 5.71 % at an EDTA:Fe molar ratio of 0.67:1. Increasing the Na<sub>2</sub>EDTA content to give an EDTA:Fe molar ratio of 1:1 did not further increase Fe absorption. The absorption ratios compared with FeSO<sub>4</sub> alone were 2.49 ( $P < 0.01$ ) at an EDTA:Fe molar ratio of 0.33:1, 5.62 ( $P < 0.001$ ) at a 0.67:1 molar ratio, and 5.51 ( $P < 0.001$ ) at a 1:1 molar ratio. With the wheat–soyabean cereal (study no. 8), Fe absorption was slightly lower and ranged from 0.70 % when fortified with FeSO<sub>4</sub> alone to a maximum of 2.86 % when Na<sub>2</sub>EDTA was added to give an EDTA:Fe molar ratio of 1:1. The absorption ratios compared with FeSO<sub>4</sub> alone were 2.52 ( $P < 0.01$ ) at an EDTA:Fe molar ratio of 0.33:1, 3.17 ( $P < 0.001$ ) at a 0.67:1 molar ratio, and 4.08 ( $P < 0.001$ ) at a 1:1 molar ratio. Increasing the EDTA:Fe molar ratio from 0.67:1 to 1:1 increased Fe absorption from 2.22 % to 2.86 %, although the absorption ratio of 1.29 was not significant ( $P > 0.05$ ).

In study no. 9 (Table 3), Fe absorption from the ferric pyrophosphate-fortified cereal was only 0.26 % compared with 1.76 % when fortified with FeSO<sub>4</sub>. As in study no. 7, adding Na<sub>2</sub>EDTA to FeSO<sub>4</sub> at an EDTA:Fe molar ratio of 1:1 significantly increased Fe absorption from 1.76 to 5.93 %, a 3.4-fold increase ( $P < 0.001$ ). Adding the same molar ratio of Na<sub>2</sub>EDTA to ferric pyrophosphate-fortified cereals resulted in a much lower increase

in Fe absorption from 0.26 up to 0.44 %, a 1.7-fold increase ( $P > 0.05$ ).

### Discussion

FeSO<sub>4</sub> is an Fe compound of high relative bioavailability in human subjects. It is usually the benchmark for comparing different Fe compounds and has been designated a relative bioavailability of 100 (Hurrell, 1997). When FeSO<sub>4</sub> is used to fortify cereal foods, however, Fe absorption by human subjects may be unacceptably low due to the natural presence of phytic acid (Cook *et al.* 1997). In the present studies Fe absorption from FeSO<sub>4</sub> by normal adults was less than 1 % from the high-phytic acid-containing wheat–soyabean and quinoa infant cereals, and from the high-extraction-wheat flour (studies nos. 2–4 and 6; Table 1). Fe absorption was only approximately 2 % from the FeSO<sub>4</sub>-fortified wheat infant cereal made from low-extraction-wheat flour, but increased to approximately 6 % when the same flour was baked into a bread roll, presumably due to the degradation of phytic acid during our bread-making process due to the activation of phytases. As would be expected, percentage Fe absorption increased as the Fe content of the meals decreased, being highest from the unfortified meals and lowest from the meals containing 15 mg fortification Fe.

Ferrous fumarate is an alternative Fe-fortification compound to FeSO<sub>4</sub>. It has been reported to have the same relative bioavailability as FeSO<sub>4</sub> in adults (Hurrell *et al.* 1989). Its main advantage is that it causes few, if any, colour and flavour changes in infant cereals (Hurrell *et al.* 1989), chocolate-drink powders (Hurrell *et al.* 1991) and maize flour (Layrisse *et al.* 1996). Unfortunately, it is not protected from phytic acid like FeSO<sub>4</sub>, and can be poorly absorbed when added to cereal foods. In our studies, Fe absorption ranged from 0.57 to 2.06 % (studies nos. 1–3; Table 1), with an absorption ratio compared with FeSO<sub>4</sub> of 0.91–1.28.

NaFeEDTA has been suggested as an ideal Fe fortificant for cereal-based foods, since the EDTA moiety protects Fe from phytic acid (International Nutritional Anemia Consultative Group, 1993) and prevents Fe-catalysed fat oxidation reactions during the storage of cereal flours (Hurrell, 1997). The present studies have shown that NaFeEDTA is a useful additive for a variety of cereal-based foods. Fe absorption was 2–4-fold greater than that from FeSO<sub>4</sub>, confirming earlier reports on the absorption of Fe from NaFeEDTA-fortified cereal foods. Viteri *et al.* (1978) have previously reported a 2.5 times greater Fe absorption by children from a rice–milk cereal when fortified with NaFeEDTA than when fortified with FeSO<sub>4</sub>, and MacPhail *et al.* (1981) similarly reported a 2-fold increase in Fe absorption by adult women from a NaFeEDTA-fortified maize porridge.

Although it is clear that EDTA enhances Fe absorption from foods containing phytic acid, our results suggest that it does not provide complete protection. Fe absorption by volunteers consuming the high-extraction-wheat bread roll fortified with NaFeEDTA (3.91 %; Table 1) was much lower than from the low-extraction-wheat roll (11.5 %) in which the phytic acid had been completely degraded, even

though the serum ferritin levels in the former group of volunteers were lower. Similarly, when fortified with NaFeEDTA, Fe absorption from the high-phytate wheat–soyabean cereal and the quinoa cereal (2.81 and 1.68 % respectively; Table 1) was lower than from the low-phytate wheat cereal (5.23 %). Earlier reports that phytic acid had little influence on Fe absorption from NaFeEDTA were based on the observation that bran added to an aqueous solution of NaFeEDTA did not influence Fe absorption, whereas adding bran to an aqueous solution of FeSO<sub>4</sub> greatly reduced Fe absorption (MacPhail *et al.* 1985).

The polyphenols from tea are also known to strongly inhibit Fe absorption (Hurrell *et al.* 1999); consuming tea with the low-extraction-wheat roll fortified with NaFeEDTA reduced Fe absorption from 11.5 % to 1.86 % (Table 1), thus confirming the earlier report that EDTA cannot completely overcome the inhibitory effect of tea (MacPhail *et al.* 1981). Although NaFeEDTA does not provide complete protection against phytic acid and polyphenols, in the presence of these inhibitory substances, Fe absorption from cereal foods fortified with NaFeEDTA is 2–4-fold higher than when fortified with Fe compounds such as FeSO<sub>4</sub> or ferrous fumarate. NaFeEDTA would thus seem an ideal compound for the fortification of cereal foods, especially as it has recently been approved as a food additive by the Joint FAO/WHO Expert Committee on Food Additives (1999). It should be remembered however that, as with other soluble Fe compounds, NaFeEDTA can cause unwanted colour reactions such as those reported in cereal products with bananas and in chocolate (Hurrell, 1997).

The purpose of the second part of the present study was to evaluate the use of Na<sub>2</sub>EDTA as an enhancer of Fe absorption. The only enhancer of Fe absorption available to food manufacturers at the present time is ascorbic acid, which has been shown to increase the absorption of soluble Fe compounds such as FeSO<sub>4</sub>, less-soluble Fe compounds such as ferric orthophosphate and elemental Fe (Forbes *et al.* 1989), and native food Fe (Layrisse *et al.* 1977). The advantage of Na<sub>2</sub>EDTA over ascorbic acid is that it is stable during food processing and storage, and earlier studies have shown that it can increase the absorption of FeSO<sub>4</sub> added to a rice meal (MacPhail *et al.* 1994).

In the present studies, we have mainly investigated the influence of EDTA on the absorption of Fe from FeSO<sub>4</sub>, a soluble Fe compound. In one study, however, we investigated the influence of Na<sub>2</sub>EDTA on Fe absorption from the insoluble ferric pyrophosphate. The addition of Na<sub>2</sub>EDTA to infant cereals fortified with FeSO<sub>4</sub> increased Fe absorption progressively as the EDTA:added Fe molar ratio was increased. In both the wheat and wheat–soyabean cereals, Fe absorption was increased at EDTA:Fe molar ratios of less than 1:1 (Fig. 1). In the wheat cereal the maximum increase in Fe absorption occurred at an EDTA:Fe molar ratio of 0.67:1, supporting the earlier report from MacPhail *et al.* (1994) that an EDTA:Fe molar ratio of 0.5:1 resulted in the maximum increase in Fe absorption from rice fortified with FeSO<sub>4</sub>. With the higher-phytate-containing wheat–soyabean cereal we found the maximum increase in Fe absorption at the EDTA:Fe molar ratio of 1:1.

In contrast to its strong positive influence on Fe absorption from wheat cereal fortified with  $\text{FeSO}_4$ , we could demonstrate no influence of  $\text{Na}_2\text{EDTA}$  on Fe absorption from the same cereal fortified with ferric pyrophosphate. Fe absorption from the cereal fortified with ferric pyrophosphate was only 0.26 % compared with 1.76 % with  $\text{FeSO}_4$  (Table 2).  $\text{Na}_2\text{EDTA}$  increased  $\text{FeSO}_4$  absorption 3.4-fold ( $P < 0.001$ ), whereas there was no increase in Fe absorption from ferric pyrophosphate. The lack of enhancing effect of  $\text{Na}_2\text{EDTA}$  on Fe absorption from ferric pyrophosphate is possibly related to the low solubility of this Fe compound in the gastrointestinal tract.

In situations where ascorbic acid is unstable during processing and storage,  $\text{Na}_2\text{EDTA}$  would appear to be a useful Fe absorption enhancer for addition together with soluble Fe compounds to inhibitory foods, or foods consumed in combination with inhibitory meals. Food products that could be considered for such fortification include beverages, milk, pasta, salt, sugar, soya sauce, fish sauce and cereal flours or weaning cereals that are stored for only short periods of time. However, in relation to Fe absorption, there would seem to be no advantage in adding  $\text{Na}_2\text{EDTA}$  plus a soluble Fe compound rather than adding  $\text{NaFeEDTA}$ .  $\text{Na}_2\text{EDTA}$  plus  $\text{FeSO}_4$  may be less expensive, but  $\text{NaFeEDTA}$  may have the advantage of causing less sensory changes. For example, it is better to add  $\text{NaFeEDTA}$  to cereal flours or weaning cereals, since  $\text{FeSO}_4$  plus EDTA, like  $\text{FeSO}_4$  alone, has been shown to catalyse fat oxidation reactions during storage (Hurrell, 1997).

A possible fortification strategy in developing countries where diets are high in both Fe and phytic acid would be to fortify foods with  $\text{Na}_2\text{EDTA}$  alone. The EDTA moiety will combine with the native food Fe that enters the common Fe pool in the gastrointestinal tract, thereby preventing the formation of non-absorbable Fe–phytate complexes and enhancing Fe absorption. This strategy would be less expensive and would avoid many of the sensory problems associated with the addition of Fe to foods.

$\text{Na}_2\text{EDTA}$  would be a major aid to Fe-fortification programmes if it enhanced Fe absorption from poorly-soluble Fe compounds such as ferrous fumarate, ferric pyrophosphate and elemental Fe. These compounds can often be added to foods when  $\text{NaFeEDTA}$  causes unacceptable sensory changes, or is considered too expensive. Although ferrous fumarate has a similar relative absorption to  $\text{FeSO}_4$ , ferric pyrophosphate and elemental Fe are much less well absorbed. The addition of  $\text{Na}_2\text{EDTA}$  to foods fortified with these compounds could potentially increase Fe absorption to nutritionally-useful levels. Although in our studies we could show no benefit of  $\text{Na}_2\text{EDTA}$  on Fe absorption from wheat cereal fortified with ferric pyrophosphate, this radiolabelled compound was only 15 % as well absorbed as  $\text{FeSO}_4$ . This value is lower than the 21–74 % relative absorption previously reported for ferric pyrophosphate in human studies (Hurrell, 1997) and it is still possible that other Fe compounds, such as ferrous fumarate, elemental Fe powders, or even different batches of ferric pyrophosphate, which are more readily dissolved in the common Fe pool in the gastrointestinal tract, will be enhanced in absorption by

$\text{Na}_2\text{EDTA}$  in a similar way to the soluble Fe-fortification compounds.

EDTA is capable of binding stoichiometrically with virtually every metal in the periodic table (West & Sykes, 1960).  $\text{Fe}^{3+}$  binds EDTA strongly at the pH of the stomach but, as the pH rises in the intestine, the bond weakens and EDTA complexes are formed with other metals (International Nutritional Anemia Consultative Group, 1993). Which metal complex is formed depends on the binding constant of the metal with EDTA, the pH and the EDTA:metal ion molar ratio (West & Sykes, 1960). There is a concern that EDTA compounds added to foods may negatively influence the metabolism of other essential minerals, such as Zn, Ca, Mg and Cu, or increase the absorption of potentially-toxic minerals such as Mn, Pb, Hg, Al and Cd. Studies with the nutritionally-important minerals indicate a positive influence of EDTA or no effect.  $\text{NaFeEDTA}$  has been shown to increase Zn absorption in rats and adult women, easily compensating for an increased urinary Zn excretion (Davidsson *et al.* 1994b; Hurrell *et al.* 1994), whereas  $\text{NaFeEDTA}$ -fortified bread rolls fed to women had no influence on Ca absorption or urinary excretion (Davidsson *et al.* 1994b). There are no human studies reporting the influence of EDTA compounds on Mg or Cu absorption. There are also virtually no studies on the influence of EDTA compounds on the absorption and metabolism of potentially-toxic minerals. Davidsson *et al.* (1998) showed that consumption of an  $\text{NaFeEDTA}$ -fortified infant cereal by adults had no influence on Mn absorption and urinary excretion. However, there is still a need to evaluate the influence of EDTA compounds on the metabolism of Pb, Hg, Cd and Al.

In summary, we have demonstrated that  $\text{NaFeEDTA}$  is a useful Fe fortificant for cereal-based foods. Fe absorption by human subjects was 2–4-fold greater from infant cereals or bread rolls fortified with  $\text{NaFeEDTA}$  than from the same foods fortified with either  $\text{FeSO}_4$  or ferrous fumarate. We have also demonstrated that  $\text{Na}_2\text{EDTA}$  will enhance the absorption of  $\text{FeSO}_4$  at EDTA:Fe molar ratios of 1:1 and below, indicating that  $\text{Na}_2\text{EDTA}$  can be used as an alternative to ascorbic acid to enhance the absorption of soluble Fe compounds as well as native food Fe. We could not demonstrate, however, that  $\text{Na}_2\text{EDTA}$  will enhance the absorption of insoluble Fe compounds such as ferric pyrophosphate.

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